Characterizing the CSF Biomarker Signature of Calpain-2 Activity in ALS and Its Application in the Phase 1 LUMINA Trial

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mnda motor neurone disease association
36th international symposium on ALS/MND

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Disclosures

These studies were sponsored by Amylyx Pharmaceuticals, Inc.

Disclosures

RB and **JA** are employees of *n*Vector, Inc.

LK, **EM, EB,** and **JT** are employees of and have stock option ownership in Amylyx Pharmaceuticals, Inc.

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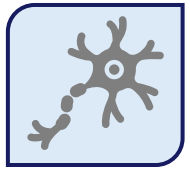
Please Note

- AMX0114 is an investigational drug and has not been approved by any health authority.
- This presentation is intended to provide scientific information about AMX0114. The statements and content shared in this presentation have not been evaluated by any health authority.

Presentation Overview



Calpain-2 and Axonal Degeneration in ALS

Calpain-Driven Biomarkers: SBDP-145 Assay Qualification and CSF Characterization in ALS

Calpain-Driven Biomarkers: Emerging Insights

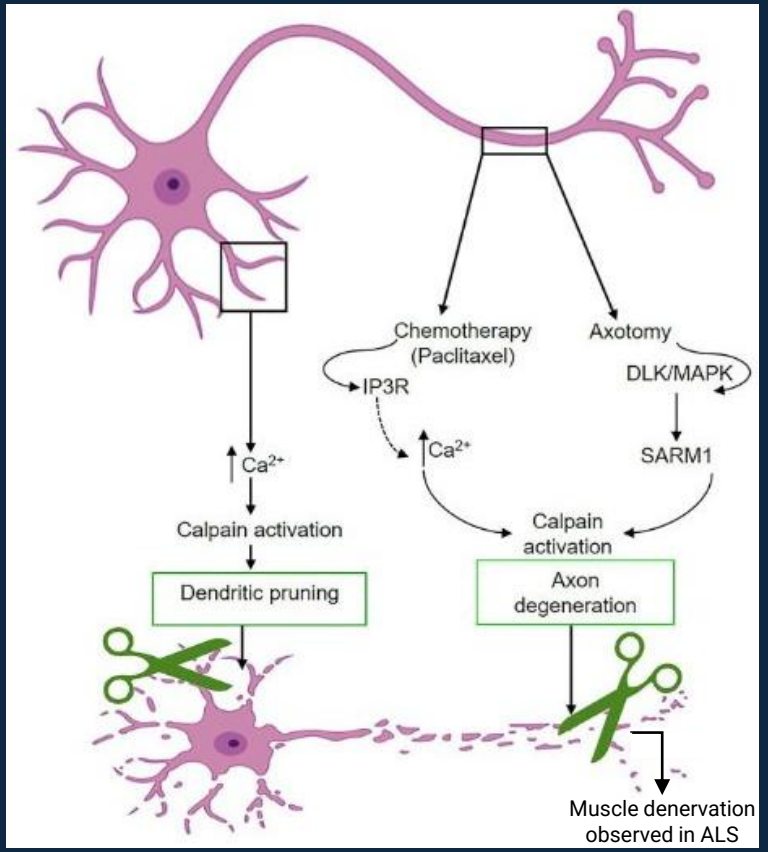
Calpain-2 and Axonal Degeneration in ALS

Calpain-2, a Ca^{2+}-Activated Cysteine Protease, Drives Axonal Degeneration and Contributes to the Pathogenesis of ALS

Importance of Calpain-2 in ALS

- **Effector of Axonal Degeneration**[1-6]
  Calpain-2 is a calcium-dependent protease that drives early axonal degeneration by cleaving cytoskeletal proteins such as αII-spectrin and NfL
- **Mechanistic Link to ALS Pathways**[8-11]
  Calpain-2 contributes to excitotoxicity, mitochondrial dysfunction, neuroinflammation, and disrupts nucleocytoplasmic transport — key intersecting mechanisms in ALS pathogenesis
- **Therapeutic Target**[12]
  Calpain-2 is targeted by AMX0114, a novel antisense oligonucleotide (ASO) that shows neuroprotective effects in disease cell models

Mechanisms of Axonal Degeneration[13]

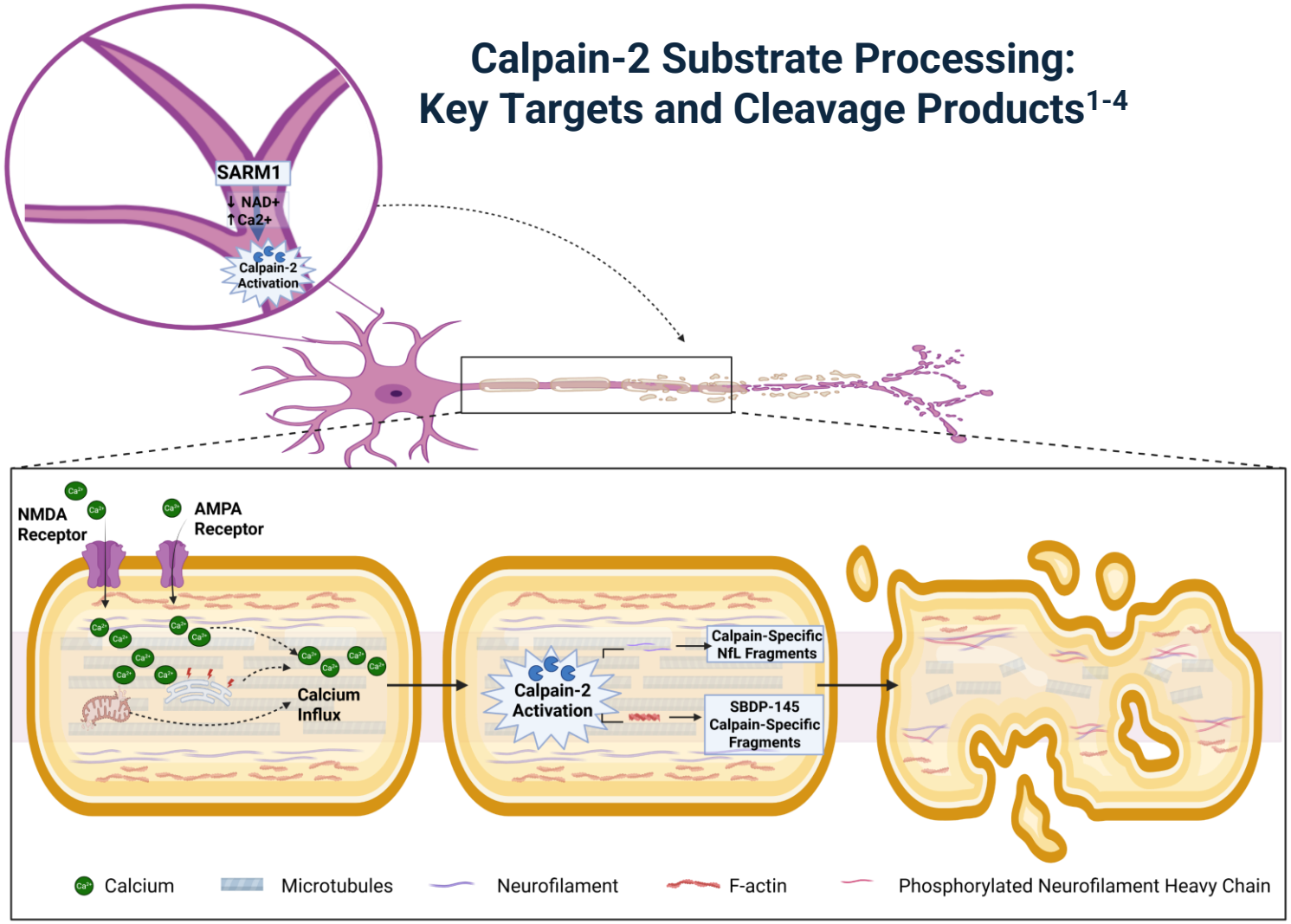


Multiple injury paradigms and hypotheses of axonal degeneration converge on calpain-2

1. Moloney EB, et al. *Front Neurosci*. 2014;8:252. **2**. Ma M. *Neurobiol Dis*. 2013;60:61-79. **3**. Asakawa K, et al. *Cell Mol Life Sci*. 2021;78(10):4453-4465. **4**. Ueyama H et al. *J Neurol Sci.* 1998;155():163-169. **5.** Yamashita, T et al. *Nat Commun*. 2012; 3:1307. **6.** Rao MV et al. *J Neurochem.* 2016;137(2):253-265. **7**. Wang Y, et al. *Cells*. 2020; 9(12): 2698. **8.** Tadic V, et al. *Front. Cell. Neurosci*. 2014; 8. **9.** Yamashita T et al. *Sci Rep.* 2017; Jan 3;7:3999. **10.** Knaryan VH and Sarukhanyan FP. *Neurosci Behav Physiol.* 2024; 54(1):27-34. **11.** Smith MA and Schnellmann RG. *Cardiovascular Research.* 2012; 96(1):32-37. **12.** Mizerak E, et al. Presented at the TIDES USA Conference; Boston, MA; May 14-17, 2024. **13.** Metwally E, et al. *Front. Vet Sci.* 2023;10:1235163.

Calpain-Specific Substrates Include Alpha II-Spectrin and NfL

Calpain-2 Substrate Processing: Key Targets and Cleavage Products[1-4]



Calpain-2 cleaves cytoskeletal proteins alpha II-spectrin and NfL resulting in breakdown products that may serve as markers of calpain-2 activity:[1,5-8]

- **SBDP-145:** calpain-specific alpha II-spectrin breakdown product
- Calpain-induced **NfL fragments**

1. Yamashita T, et al. Nat Commun. 2012; 3:1307. **2**. Tadic V, et al. Front. Cell. Neurosci. 2014; 8. **3.** Loring HS, et al. Cell Chem Biol. 2020; 27(1):1-13. **4.** Miyamoto T, et al. J Biol Chem. 2024; 300(2):105630. **5.** Ma M. Neurobiol Dis. 2013;60:61-79. **6.** Wang Y, et al. Cells. 2020; 9(12): 2698. **7.** Ono Y, Saido TC, Sorimachi H. Nat Rev Drug Discov. 2016;15(12):854-876. **8.** Ma M, et al. Neurobiol Dis. 2013;56:34-46.

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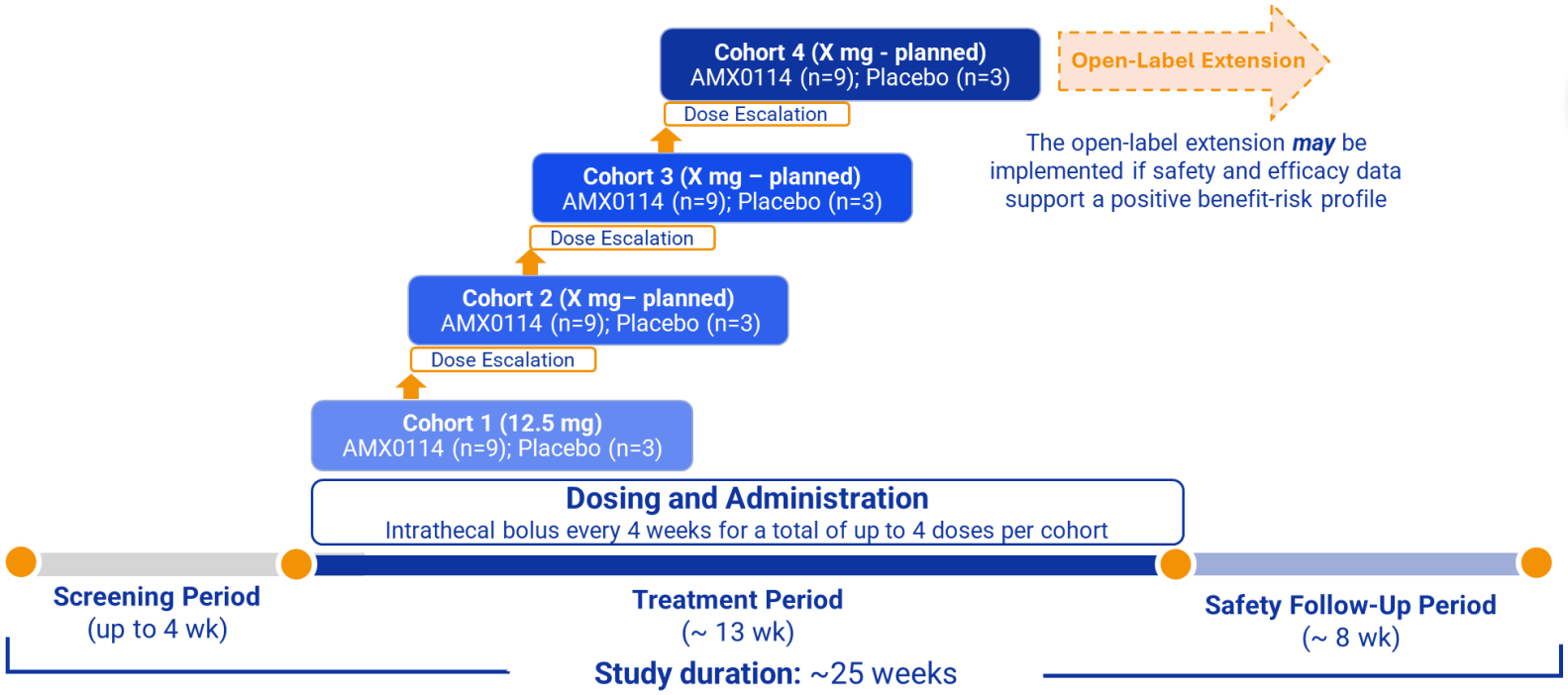
LUMINA A Phase 1, Multicenter, Randomized, Placebo-Controlled Multiple Ascending Dose Trial of AMX0114, an ASO Targeting Calpain-2

Study Objectives:

- Evaluate the safety and tolerability of AMX0114 administered intrathecally for a total of up to 4 doses per dose level
- Evaluate the pharmacokinetics of AMX0114 in CSF and plasma
- Assess the effects of AMX0114 on plasma and CSF biomarkers and functional measures of ALS disease progression

LUMINA Trial Design and Tertiary Endpoints



Tertiary Endpoints

Pharmacodynamics/Biomarkers

- Change from baseline of plasma and CSF pharmacodynamic measures of ALS and markers of target engagement (e.g., calpain-2 levels, NfL, SBDP-145)

ALS Progression Measures

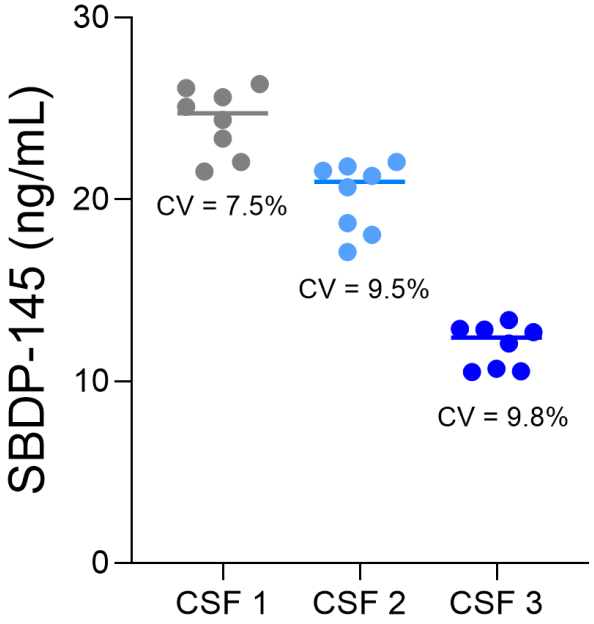
- Change from baseline of ALS Functional Rating Scale – Revised (ALSFRS-R) and slow vital capacity (SVC)

Calpain-Driven Biomarkers:

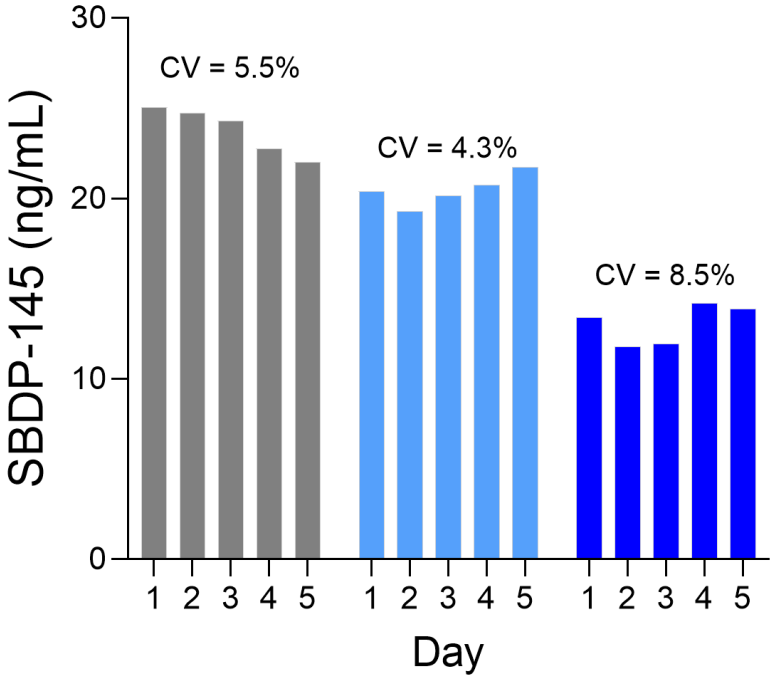
SBDP-145 Assay Qualification and CSF Characterization in ALS

SBDP-145 Assay Qualification Ensures Precision, Accuracy, and Dilutional Linearity

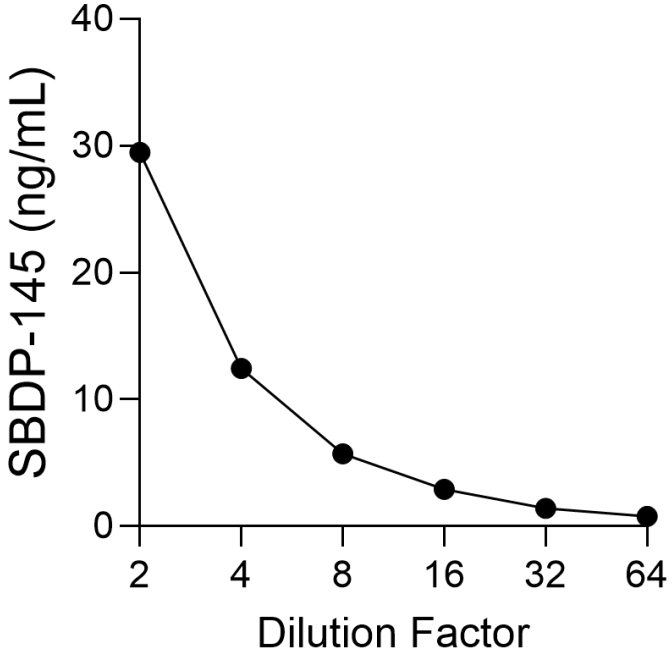
Intra-Assay Precision of endogenous SBDP-145 from human ALS CSF samples



Inter-Assay Precision of endogenous SBDP-145 from human ALS CSF samples



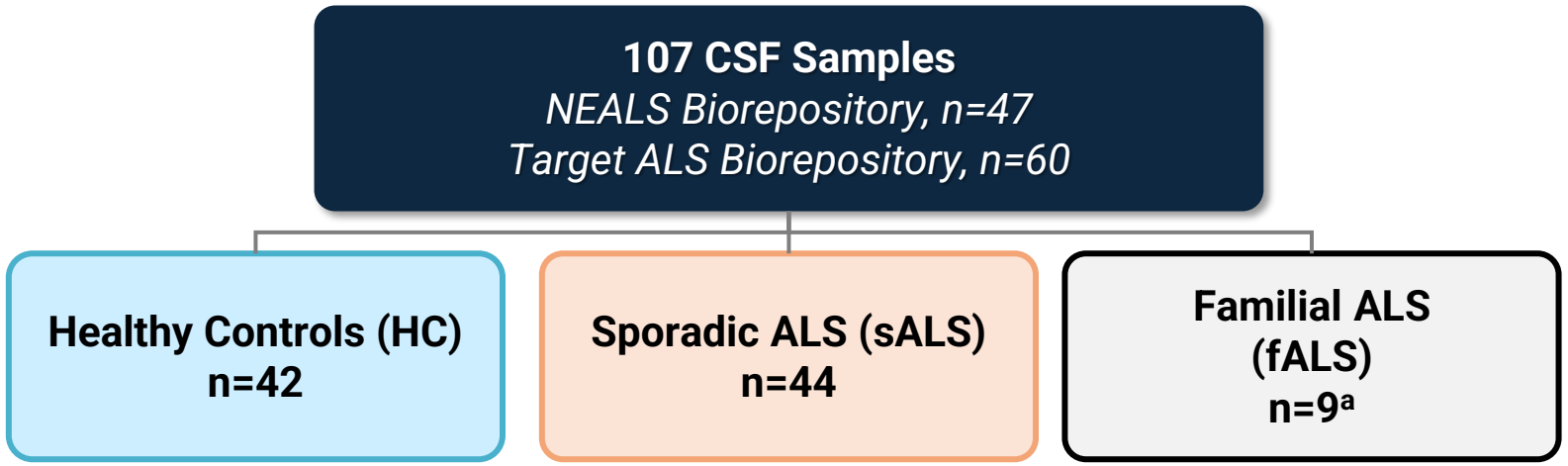
Dilutional Linearity with a high spike at 60 ng/mL met acceptance through a dilution factor of 64



CSF 1 CSF 2 CSF 3

Measuring Levels of SBDP-145, a Calpain-Specific Breakdown Product, in ALS CSF

Methods

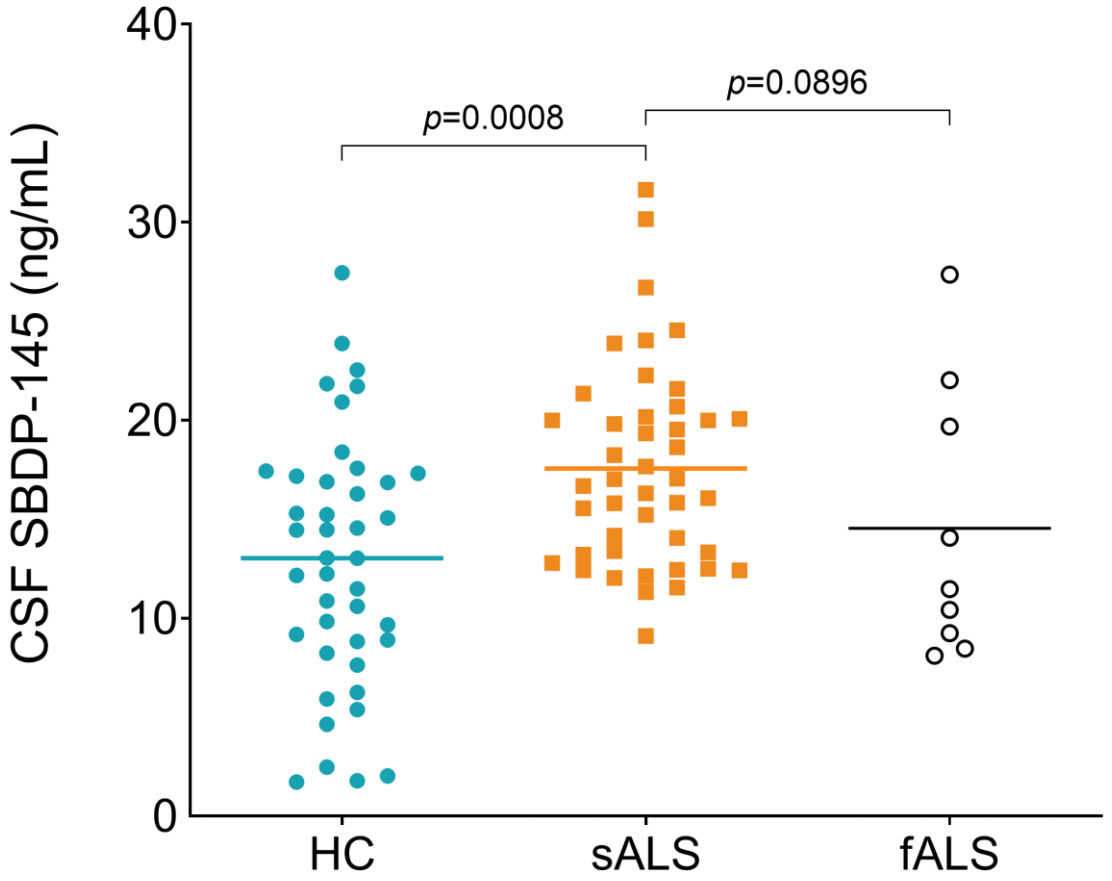


[a]7 C9orf72, 1 SOD1, and 1 TAF15 mutation carriers

Assay Details

- CSF SBDP-145 level quantification using ELISA (Biomatik)
- All samples run in duplicate
- Standard curves (0-40 ng/mL) generated per plate
- Individual values and median calculated per diagnostic group

Levels of SBDP-145, a Calpain-Specific Breakdown Product, Were Significantly Higher in CSF From People Living with ALS Compared to Controls



HC: Healthy Controls
sALS: Sporadic ALS
fALS: Familial ALS

Levels of SBDP-145 were significantly higher in CSF from people living with ALS (combined sALS and fALS) compared to controls (***p*=0.0029**)[1]

1. Bowser R, et al. Presented at the 2025 NEALS Annual Meeting; Clearwater Beach, Florida; October 7-10, 2025.

Calpain-Driven Biomarkers: Emerging Insights

NfL in ALS: Key Biomarker, But Assay Gaps Remain

NfL is a Well Described ALS Biomarker

- NfL levels are increased in people living with ALS, measured by immunoassays[1-3]
- NfL levels increase early in disease and stay relatively constant over time[2-4]
- Calpain activity can lead to NfL cleavage[5-7]

NfL Immunoassay Challenges

NfL assays likely detect fragments rather than full-length protein[8-10]. Literature suggests full-length NfL is undetectable in CSF, and the ALS signal is potentially driven by fragments, which remain poorly characterized.

- Ongoing assay development will **characterize NfL fragments in CSF**
- **Calpain-induced NfL fragment** identification will further inform **AMX0114 target engagement**

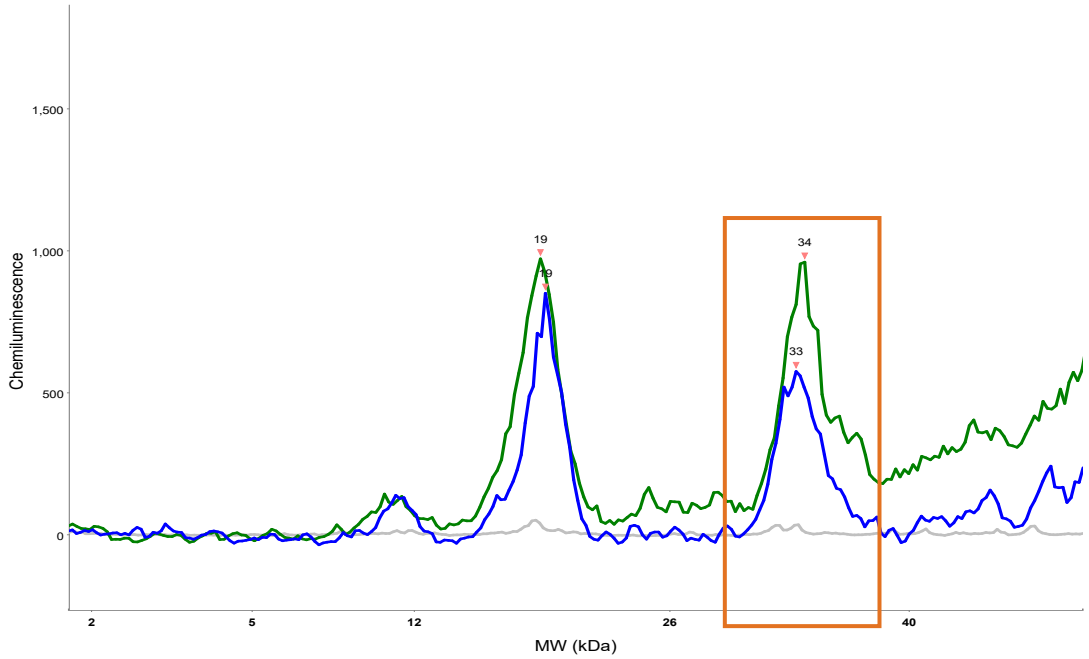
NfL: Neurofilament Light Chain

1. Lu C, et al. *Neurology*. 2015;84(22):2247-2257. **2.** Vacchiano V, et al. *Front. Aging Neurosci.* 2021;13. **3.** Verde F, et al. *Journal of Neurology, Neurosurgery & Psychiatry*. 2019;90:157-164. **4.** Benatar M, et al. *Brain.* 2022;146(7):2711-2716. **5.** Kahn O, et al. *Cell Reports*. 2025;44(3):115382. **6.** Ma M. Neurobiol Dis. 2013;60:61-79 **7.** Becker B, et al. *Brain Communications.* 2025;7(2):fcaf129. **8.** Budelier M, et al. *Brain Communications.* 2022;4(2):fcac045. **9.** Meda F, et al. *BMJ Neurology Open.* 2023;5:e000395. **10.** Becker B, et al. *Brain Communications.* 2025;7(2):fcaf129.

NfL Fragment Characterization: 34 kDa Fragment Enriched in ALS CSF and Calpain-Induced Fragments of Recombinant NfL (rNFL) Using Jess Simple Western

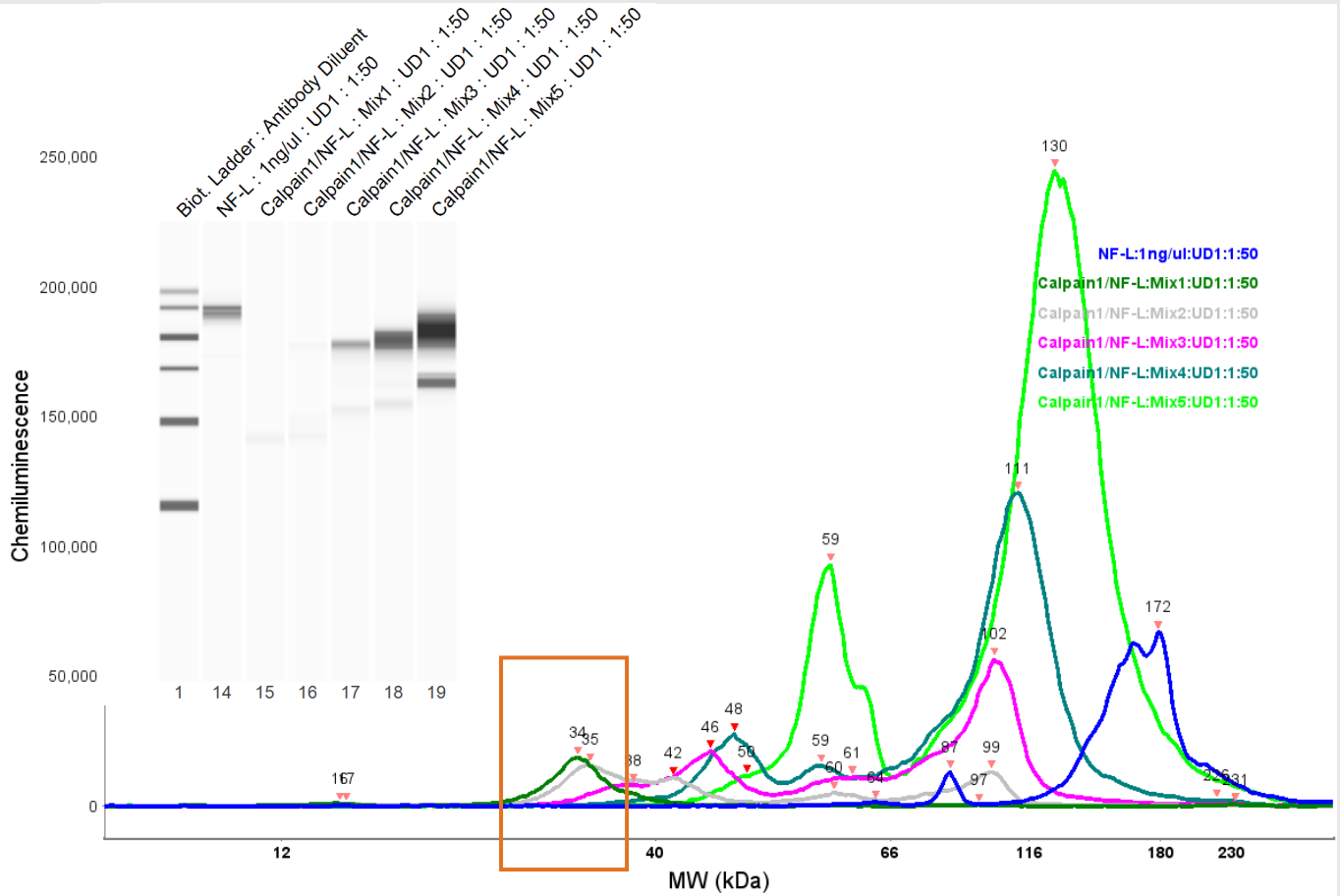
19 and 33-34 kDa NfL Fragments Are Present in ALS CSF Among Other Major Bands

2 NfL antibodies confirm fragments of 19 and 33-34 kDa



Other major bands present at 55, 58-60, 76-78, 141-142 kDa

Calpain-Induced Fragments of rNfL



rNfL calpain cleavage fragments of 16 kDa, 34/35 kDa and higher MW species are observed. Confirmed with Jess 2-40 kDa capillary electrophoresis system.

Key Takeaways

Calpain-2 is a critical effector of axonal degeneration, a key early contributor to the pathogenesis of amyotrophic lateral sclerosis

SBDP-145 is a calpain-specific breakdown product and serves as a biochemical marker of neuronal injury

SBDP-145 is elevated in CSF from people living with ALS – supporting the role of SBDP-145 as a biomarker of calpain-2 activity, axonal degeneration, and AMX0114 target engagement

Calpain-induced fragments of rNfL are of similar MW as NfL fragments detected in ALS CSF. Ongoing work aims to further define these fragments for potential use as biomarkers of AMX0114 activity

QUESTIONS?

LUMINA

Scan to Learn More about the LUMINA Trial